sulphate and that can be separated from the glycosulphatase by fractionation with ammonium sulphate (Lloyd & Fielder, 1967). The nature of this second enzyme has now been more closely defined.

The substrate used was chondroitin 4-sulphate from bovine trachea, which had been freed from protein by successive digestions with trypsin, papain and Pronase, and fractionally precipitated from ethanol. Incubation of the chondroitin 4-sulphate with the enzyme preparation led not only to the liberation of inorganic sulphate but also to the concurrent production of diffusible reducing saccharides, indicating that a polysaccharase was also present. Both sulphatase and polysaccharase were inhibited by borate and phosphate ions, but, whereas polysaccharase activity was completely inhibited by cyanide ions, sulphatase activity was slightly increased. From the chondroitin sulphate-enzyme-CN⁻ digest was isolated, after deproteinization and dialysis, in quantitative yield (compared with a control), a modified chondroitin sulphate of lower electrophoretic mobility, showing diminished metachromasia with toluidine blue. That desulphation of the polysaccharide had occurred was confirmed by analysis, which indicated a 37% decrease in ester sulphate content. Thus it is established that, unlike the 'chondrosulphatase' of *Proteus vulgaris* (Dodgson & Lloyd, 1957), this enzyme is capable of desulphating chondroitin sulphate without prior depolymerization. Sulphate was also liberated by the enzyme from a range of chondroitin sulphates from other sources, including a commercial preparation believed to be predominantly chondroitin 6-sulphate.

The activity of the enzyme towards one more substrate was determined. This was a mixture of oligosaccharides (mainly tetrasaccharide) prepared by depolymerization of chondroitin 4-sulphate with testicular hyaluronidase, the product being isolated after deproteinization and desalting by analysis on Sephadex G25 (Flodin, Gregory & Rodén, 1964). The enzyme desulphated this substrate at a rate comparable with that recorded with the parent polysaccharide. Studies on optimum conditions for reaction and on mixed substrates suggest that a single enzyme, of wide specificity in respect of molecular size, may be responsible for the desulphation of both substrates.

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The Incorporation of L-[³H]Lysine into the Histone Fractions of Rat Thymus *in vivo*

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Previous studies by Byvoet (1966) and Piha, Cuenod & Waelsch (1966), on a variety of tissues, have demonstrated that the relative rates of turnover of DNA and histones are approximately the same. The relative rates of turnover of the individual histone fractions, however, appear to be the subject of some controversy (for review, see Hnilica, 1967). We have therefore investigated the relative incorporation of L-[³H]lysine into the main histone fractions of rat thymus. In one experiment ten rats were injected intraperitoneally with L-[³H]lysine (0.5 mc/rat) and killed after 24 hr. The four histone fractions, F1, F2(a), F2(b), and F3, were then isolated from the thymus glands as described previously (Johns, 1964). All the fractions were subjected to amino acid analysis and polyacrylamide-gel electrophoresis. The analyses and gel patterns were similar to those reported previously for calf thymus (Johns, 1964, 1967a). The relative radioactivities per unit weight of protein for the various fractions, corrected for lysine content, were: fraction F1, 1420; fraction F2(b), 1450; fraction F2(a), 1450; fraction F3, 1500. In a second similar experiment, the F2(a) group was selectively extracted from the saline-washed rat thymus and separated into its two components, fractions F2(a)1 and F2(a)2 (Johns, 1967b). Relative radioactivities per unit weight of protein, corrected for lysine content, were 1300 for fraction F2(a)1 and 1320 for fraction F2(a)2. The relative rates of incorporation of L-[³H]lysine into these two subfractions have not previously been investigated and, since they also are very similar, it would appear that all the five major histone fractions of rat thymus turn over at the same rate.

Similar results were also obtained by the following method. Labelled whole histone from rat thymus was separated on polyacrylamide gel (Johns, 1967a). The bands corresponding to individual fractions were sliced from the stained gel, and the labelled protein was extracted with 6N-HCl at 90°. The HCl was removed by evaporation before the residue was dissolved in water and counted in a liquid-scintillation counter. The protein content of each band was determined quantitatively on parallel gels by the method previously described (Johns, 1967a).

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The Effect of Excess of Dietary Lysine on Chick Liver Transaminidase

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Lysine, when given in dietary excess to chicks, leads to arginine deficiency (Smith & Lewis, 1966). The development of signs of this condition, e.g. depressed growth rate and lowered plasma and muscle free arginine concentrations, apparently varies with the dietary protein source (Jones, 1964). The enzyme transaminidase, requiring arginine as substrate, effects the initial stage in creatine formation.

A complete diet (diet 1), based on whole egg protein, and a similar diet (diet 2), equinutritive with diet 1 after the addition of 2.2% of L-lysine, were compounded. Each diet was fed to a group of 14-day-old male chicks for 15 days. The depression in growth rate with diet 2, together with the twofold increase in the plasma concentration and excretion of uric acid, suggests poor protein utilization of this diet compared with diet 1.

At the end of the experimental period, liver transaminidase was assayed (Walker, 1960); diet 2 caused a decrease of one-third in enzyme activity

compared with diet 1. A decrease was also reported by Jones, Petersburg & Burnett (1967) under similar experimental conditions.

The free amino acid concentrations of individual plasma, liver and leg-muscle samples were determined by ion-exchange chromatography. Lysine was increased in plasma, liver and muscle of the chicks on diet 2, whereas liver arginine was decreased. The plasma and muscle arginine concentrations showed no significant difference with the dietary treatment, an observation that differs from previous reports (Jones, 1964; Jones *et al.* 1967). In this study considerable individual variation in muscle free arginine concentrations was found within each group.

The decrease in transaminidase activity with diet 2 was not accompanied by a significant change in liver creatine; the increase in leg-muscle creatine was only marginally significant. The indications of this study add weight to the comment of Walker & Wang (1964) that end-product repression may not be the only factor governing tissue transaminidase. The decreased activity obtained with diet 2, in which the artificial amino acid imbalance renders the diet of low nutritive value, is in agreement with the observation of Kean (1967) that transaminidase of rat kidney increases with the nutritive value of the dietary protein.

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